Original Article

# Cytokine Genetic Polymorphism of Interleukin-4 and Risk of Asthma in Some Iraqi Patients

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## Abstract

**Background:** Asthma, a chronic inflammatory respiratory disorder, is influenced by genetic and environmental factors. Allergic asthma is becoming more common because of higher levels of air pollution. Interleukin-4 (IL-4) plays an important role in allergic inflammation and causes the expression of vascular cell adhesion molecule-1. **Objective:** The present study aims to shed light on the association between cytokine genetic polymorphisms and asthma in Iraqi patients, and to determine their impact on the risk of disease, under the scope of the following: (1) IL-4 cytokines, in terms of their serum level. (2) Assessment of the polymorphisms in the promoter regions of the IL-4, genes in asthma disease patients was carried out, and then their impact on the profile of investigated cytokines was evaluated. Such a collective evaluation may aid in a better understanding of etiopathogenesis in the asthma disease. **Materials and Methods:** A case-control study was conducted that included 100 participants divided into a patient group ($n$ = 50) with bronchial asthma and a healthy group as a control ($n$ = 50) without asthma. Blood specimens were collected from participants at Marjan Hospital, Babylon Governorate. Serum levels of IL-4 were estimated by an enzyme-linked immunosorbent assay (ELISA) kit. $IL4_{-590}$ gene polymorphism was detected using amplification refractory mutation system polymerase chain reaction (PCR) with specific primer sequences. **Results:** The mean concentrations of serum IL-4 were significantly higher in patients having asthma compared to the healthy group (149.84 vs. 53.50, $P < 0.0001$). In terms of allele and genotype frequencies, the TT genotype was shown to be less common in asthma group compared to controls (4% vs. 22%), whereas the CT genotype was more common in asthma patients (34% vs. 16%). The CC genotype was similar between the two groups. The T allele was more frequent in healthy (39%) compared to asthmatic group (12%), whereas the C allele frequency was higher in asthma patients (61%) compared to controls (88%). **Conclusion:** This study suggests that gene polymorphism of $IL4_{-590}$ is associated with risk of developing asthma. Asthma patients have elevated levels of IL-4 and a lower frequency of the TT genotype, indicating an increased likelihood of developing asthma when carrying the T allele and TT genotype. Conversely, the C allele may have a protective influence against asthma development.

**Keywords:** Asthma, amplification refractory mutation system-PCR, IL-4, $IL4_{-590}$ genepolymorphism

## Introduction

Asthma can be defined as a chronic inflammatory disorder affecting the respiratory system. It causes wheezing, difficulty breathing, chest tightness, and coughing due to airflow obstruction. Asthma's complexity arises from a mixture of hereditary and environmental factors impacting its severity and treatment response. It poses a significant global health challenge.[1,2] Allergic asthma prevalence is increasing worldwide due to rising air pollution levels and environmental irritants.[3] The disease involves interactions between respiratory epithelium, innate immune system, and adaptive immunity leading to chronic inflammation. Inflammatory cells release mediators that perpetuate inflammation, causing structural changes in the airways.[4]

Cytokines are extracellular signaling proteins generated through a diversity of cell classes and show critical roles in cell-to-cell communication, primarily impacting neighboring cells.[5,6] In asthma cases, cytokines are

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Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_816_23

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**Submission:** 22-Jun-2023 **Accepted:** 14-Sep-2023 **Published:** 17-Jul-2024

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**How to cite this article:** Naif AJ, Ibraheam IA. Cytokine genetic polymorphism of interleukin-4 and risk of asthma in some Iraqi patients. Med J Babylon 2024;21:359-63.

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integral to the organization and persistence of the chronic allergic inflammatory process. The term interleukin (IL) encompasses various cytokines produced by leukocytes.

IL-4 plays a crucial role as a cytokine in the progression of allergic inflammation. According to Coffman *et al*.,[7] IL-4 is associated with the stimulation of the ε isotype switch and the release of IgE by B lymphocytes. Furthermore, IL-4 demonstrates a significant function in promoting cellular inflammation within asthmatic lungs, primarily through its capacity to initiate the expression of vascular cell adhesion molecule-1 on vascular endothelium, as highlighted by Moser *et al*.[8] In this manner, IL-4 contributes to the intricate mechanisms underlying allergic reactions and asthma pathogenesis, emphasizing its pivotal importance in these processes.

The primary objective of this investigation is to examine any probable associations between $IL4_{-590}$ gene polymorphisms and serum IL-4 levels, as well as to determine their impact on an individual's susceptibility to developing asthma.

## Materials and Methods

### Patients and control

The study was carried out on 50 Iraqi with asthma patients who were referred to the Consultant Clinic at the Department of Respiratory, Marjan Teaching Hospital during the period from November 2022 until the end of December 2022. Their age range was (30-70) years. A questionnaire was designed with different questions including duration of asthma disease, family history, smoking, usage of drugs, drug duration, and controls group.

A specialist respiratory personnel collected blood samples from patients who are diagnosed with asthma by doctor who specialized in respiratory disease through clinical diagnosis and lung function test, documenting their age, gender, and treatment received. Additionally, a questionnaire form was used to gather information on age, gender, family history, education, history of lung diseases, and treatment types.

Blood samples were collected via venipuncture using disposable syringes under aseptic procedures. Five milliliters of venous blood were drawn from each participant into sterile tubes. Each blood sample was then divided into two portions: three milliliters deposited into a sterile tube with ethylenediaminetetraacetic acid (EDTA) for DNA extraction and two milliliters transferred into a plain tube for serum separation.

Serum separation involved centrifugation at 4000 rpm for 15 min to isolate serum from other components in the sample. The resulting serum samples were suitable for further analysis.

### Methods

#### *IL-4 estimation*

The levels of IL-4 in each participant's serum samples were estimated using the enzyme-linked immunosorbent assay (ELISA) technique with an ELISA kit from Monobind (Lake Forest, California, USA).

#### *DNA extraction*

The extraction of DNA was executed according to the instructions provided by Favorgen DNA extraction company kit.

#### *IL-4 (590) gene polymorphism*

The $IL4_{-590}$ gene polymorphism was detected by the amplification refractory mutation system polymerase chain reaction (PCR). Two tubes were prepared for each sample, with one tube containing both the common and reverse primers, and the other tube containing only the forward primer. The reaction mixture volume was 25 µL, comprising of 12.5 µL of master mix, 7 µL of DNA sample, 0.7 µL each of forward and reverse primers [Table 1], and 4.1 µL of distilled water.

The amplification of DNA samples was performed using a thermocycler device according to the specific PCR program detailed in Table 2 provided alongside this information.

### Statistical analysis

Statistical analyses were done using Statistical Package for the Social Sciences (SPSS; IBM, Armonk, New York, USA) version 23. Descriptive statistics were used to summarize participant characteristics. The *t* test was employed to compare IL-4 levels between the patient group and healthy control group. Logistic regression was used to calculate odds ratios and confidence intervals to assess the connection linking IL4-590 gene polymorphism and asthma risk in Babylon Governorate.

### Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and

**Table 1: Set of primes used in the study**

| Primer | Sequence | Reference | Product size (bp) |
|---|---|---|---|
| Reverse | 5′-GAATTTGTTAGTAATGCAGTCCTCC-3′ | Hussein & Jaber (2017) | - |
| T allele | 5′-ACACTAAACTTGGGAGAACATTGTT-3′ | Hussein & Jaber (2017) | 216 |
| C allele | 5′-ACACTAAACTTGGGAGAACATTGTC-3′ | Hussein & Jaber (2017) | 248 |

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analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 1434 dated October 24, 2022.

## Results

Table 3 displays the distribution of patients infected with Asthma categorized by age, for the patients group, out of a total of 50 individuals, the average age was found to be 40.16 ± 11.19832 years. Similarly, for the control group consisting of 50 individuals, their mean age was determined to be 38.34 ± 11.51505 years. The corresponding *P*-value between groups is 0.425.

The results shown in Table 4 indicate that serum IL4 levels in individuals with asthma were considerably elevated compared to the levels detected in the control group. This observation was statistically significant ($P < 0.0001$), indicating that there is a strong association between asthma and elevated serum IL4 levels.

**Table 2: The PCR program for amplification**

| No. | Steps | Temperature (ºC) | Time | No. of cycles |
|---|---|---|---|---|
| 1 | Initial denaturation | 94 | 4 min | 1 |
| 2 | Denaturation | 94 | 20 s | 38 |
| 3 | Annealing | 61 | 45 s | |
| 4 | Extension | 72 | 21 s | |
| 5 | Final extension | 72 | 2 min | 1 |

PCR: polymerase chain reaction

**Table 3: Distribution of patients infection with Asthma according to the age**

| | *N* | Mean | Std. Deviation | *P*-value |
|---|---|---|---|---|
| Patients | 50 | 40.1600 | 11.19832 | 0.425 |
| Control | 50 | 38.3400 | 11.51505 | |

**Table 4: Mean levels of IL-4 in patients infect asthma and control**

| | *N* | Mean | Std. Deviation | *P* value |
|---|---|---|---|---|
| Patients | 50 | 149.8460 | 23.67186 | <0.0001 |
| Control | 50 | 53.5040 | 21.41018 | |

Table 5 presents data related to $IL4_{-590}$ gene polymorphism, comparing patients and controls. It includes the frequency of different genotypes (TT, CT, CC), the odds ratio, confidence intervals (95%), and *P*-values.

Figures 1 and 2 show the agarose gel electrophoresis of PCR product for C allele and T allele, respectively.

## Discussion

Asthma stands for a medical disorder that can distress individuals of any time of life, and its severity can range from displaying a significant amount of symptoms to experiencing mild or even no noticeable symptoms at all.

IL-4 is a cytokine that acting a vital function in managing the inflammatory cascade associated with asthma, as it oversees an extensive array of processes. These range from guiding eosinophils through chemotaxis to the

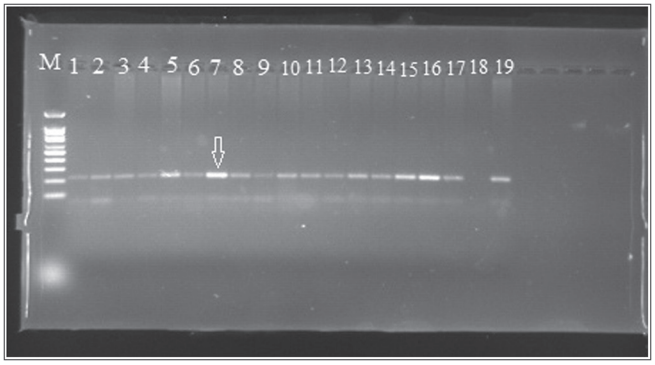


**Figure 1:** Gel electrophoresis for PCR product of C allele for IL-4 gene polymorphism. PCR = polymerase chain reaction

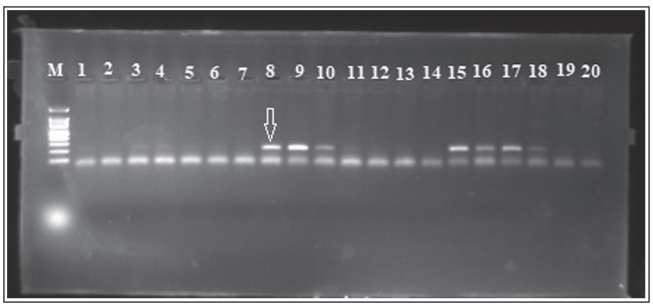


**Figure 2:** Gel electrophoresis for PCR product of T allele for IL-4 gene polymorphism. PCR = polymerase chain reaction

**Table 5: Allele and genotype frequencies of ($IL4_{-590}$) gene polymorphism between asthma patients and control patients**

| Gene polymorphism of IL4-590 | | | | | | |
|---|---|---|---|---|---|---|
| | | Patients (%) | Control (%) | Odd ratio | Confidence intervals (95%) | *P*-value |
| Genotype | TT | 11 (22) | 2 (4) | 10.0 | 2.03 to 49.23 | 0.0046 |
| | CT | 17 (34) | 8 (16) | 0.2588 | 0.0963 to 0.6954 | 0.0074 |
| | CC | 22 (44) | 40 (80) | | 1 reference | |
| Allele | T | 39% | 12% | | | |
| | C | 61% | 88% | | | |

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differentiation of naive T-helper cells into T-helper 2 cells.[9] Furthermore, IL-4 holds a vital role in modulating type two immune responses and aids in transitioning isotypes transferring from IgM/IgG to IgE in B lymphocytes. Moreover, it also helps in the employment of mast cells, which contributes significantly to inflammation within the airways.[10]

Individuals with asthma present higher levels of IL-4 compared to healthy individuals. This particular cytokine stimulates the expression of vascular cell adhesion molecule-1 on vascular endothelium, suppresses eosinophil apoptosis, and promotes eosinophilic inflammatory responses.[11] Moreover, research has demonstrated that respiratory smooth muscles cell in asthmatic persons exhibit higher levels of IL-4 contrasted to the non-asthmatic corresponding person.[12]

In additive to its other functions, IL-4 plays a crucial role in airway remodeling through its impacts on mucus-producing cells and fibroblasts.[13] The expression of mucin genes is induced by IL-4, resulting in hypersecretion of mucus from goblet cells within the airways. Researchers have found evidence suggesting that IL-4 could also act a vital function in autoantibodies' development and lupus nephritis.[14,15]

A diverse range of cells are responsible for producing IL-4, such as eosinophils, basophils, T-helper 2 cells, alveolar macrophages, and mast cells. Many cell types express the receptor of IL-4, including B lymphocytes, T lymphocytes, mononuclear phagocytes, pulmonary fibroblasts, eosinophils, endothelial cells, smooth muscle cells, and bronchial epithelial cells. The cytokine holds a crucial role in allergic airway diseases by triggering multiple transcription factors via its signaling cascade.[16]

According to the findings of the study, there appears to be a growing likelihood of developing asthma when carrying the T allele and TT genotype. It was determined that this association presents an etiological fraction with regards to risk for contracting the disease. In contrast, the control group exhibited a greater proportion of the C allele compared to individuals with asthma. This observation implies that these specific alleles might have a protective influence, which can be considered as a preventive fraction.

According to PCR analysis, the results indicated that out of the 50 patients with asthma who were tested, 22 (44%) had the CC allele while 11 (22%) had the TT allele which is considered wild type. On the other hand, 17 (34%) patients had CT allele which is classified as mutant. In contrast, in the control group consisting of 50 individuals, 40 (80%) possessed the CC allele while only 2(4%) and 8(16%) contained TT and CT alleles respectively.

Another study found that having C allele and CC genotype increases an individual's risk of developing asthma as these are associated etiological factors for this condition. Conversely, the T allele was observed to have a protecting impact versus asthma as its percentage ratio was higher among controls than in asthmatic patients. This information was reported by Hussein and Jaber[17] in their research conducted.

Additionally, Smolnikova *et al*.[18] reported that IL-4 T-590 is responsible for Excessive production of IL-4 is often linked to an unmanaged course of atopic bronchial asthma, owing to its regulatory impact on allergic inflammation. The results of the study underscore the significance of genetic factors in determining a person's vulnerability to respiratory illnesses like asthma and associated conditions.

## Conclusion

This case-control study explored the association between IL-4 gene polymorphism and asthma risk in Babylon Governorate. The findings suggest that IL-4 plays a crucial role in asthma pathogenesis due to its involvement in inflammatory processes, immune responses, airway remodeling, and mucus production. The study indicates that individuals with asthma exhibit higher levels of IL-4 compared to healthy individuals. The analysis of genotype frequencies revealed that carrying the T allele and TT genotype may increase the likelihood of developing asthma, whereas the C allele may have a protective influence.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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